FURTHER NOTES ON THE EGG OF ALLOLOBOPHORA FOETIDA.

KATHARINE FOOT AND ELLA CHURCH STROBELL.

Preface.

In the autumn of 1894, while I was studying living eggs from the ovaries of *Allolobophora foetida* with a Zeiss 2 mm. immer. lens., Dr. Whitman suggested that I kill the eggs under this high magnification in order to observe the effect of the fixatives. I made many attempts, but was unable to overcome the technical difficulties sufficiently to make the method of any value. The following spring I experimented with eggs from the cocoons; but found it impossible, without injury to the egg, to hold it steadily in the field while applying the fixative. This summer, by the aid of the Bausch and Lomb compressor, Miss Strobell and I have been able to get more satisfactory results, for it has been possible with this compressor to hold the eggs firmly and yet so gently that they continue to develop normally, forming the polar bodies, etc.

Ziegler's (10) classic work on the living Nematode eggs led me in the spring of 1896 to commence the study of the living (cocoon) eggs of *Allolobophora foetida*, and at that time I began a comparative study of the living and fixed cytoplasm of these eggs. It was my aim to be able to place side by side illustrations of the living cytoplasm with illustrations of the same stages killed by different fixatives, hoping by that method to support or correct my earlier interpretations.[1]

Since the spring of 1897 Miss Strobell has been associated with me in this work; and, as our results have been attained

[1] I quote the following from a paper sent to press December, 1897, and which will appear in *Journ. of Morph.*, vol. xiv, No. 3, 1898. "As I am at work on a paper which will give the results of a comparative study of the living and fixed cytoplasm in these eggs, I shall omit here any description of the living normal cytoplasm."

by our combined efforts, we unite in bearing the responsibility of their publication, — parts of the following paper being written by each of us. Owing to the difficulties of obtaining large numbers of these eggs at definite stages of development, it will require both time and patience to make a comparative study of much value. In the present paper we shall give a brief account of such of our results as we can support with photomicrographs; and I shall also give certain results obtained from a further study of the deutoplasm of these eggs, some data bearing on the formation of the vesicles between the first and second maturation spindles, and a few notes on shrinkage.

With orange-methyl green I had differentiated the following structures in the cytoplasm (6), the network and archoplasm (polar-ring substance) selected the orange, while the nucleoli, sperm-granules, centrosomes, and the large and small granules in the spindle, attraction spheres, and cytoplasm selected the methyl green. I did not suspect that many of these granules might be deutoplasmic, for at that time I was fully convinced that xylol or xylol balsam would, in all cases, dissolve out the deutoplasmic granules in a few hours. Further investigation showed me that the time required to dissolve the deutoplasmic granules is very inconstant. In some cases it will require days, again it will require as many weeks, and in a few cases they form an insoluble compound with the stain, and cannot be dissolved at all — this last being true even when the sections have been subjected to *exactly* the same technique, in the one case the deutoplasmic granules staining weakly and readily dissolving out, and in the other staining deeply and remaining insoluble. These facts led me to suspect their identity with many of the above-mentioned methyl-green granules, and I am greatly indebted to Miss Strobell for assisting me in my experiments to determine this point.

KATHARINE FOOT.

To test the surmise that some of the granules differentiated with methyl green were identical with the deutoplasmic granules, a number of eggs were submitted to the following technical tests. The eggs were killed in chromo-acetic, as this fixative

shows a definite structure of the cytoplasm and an approximately definite distribution of the granules; osmic acid was then used, not as a fixative, but as a stain which might select the deutoplasmic granules alone. After a few minutes in 1% osmic, the eggs were hardened, imbedded, sectioned, and mounted in glycerine without further staining. An examination of the sections showed the granules intensely blackened, and as they were apparently the only constituents in the cell that responded to the osmic, we have designated them osmophile granules (Photo. 12 and Text-fig. I). The nucleoli in the pronuclei, and when present throughout the cytoplasm, were not blackened by the osmic, and the centrosomes, which were occasionally visible in the unstained sections, were a brownish-yellow. With the diaphragm open, the intensely black osmophile granules are most sharply differentiated from the centrosomes and the greenish refractive nucleoli. Photographs were taken of many of these sections, and numerous camera sketches were made, and a comparison of these with the sketches previously made of the granules that had been differentiated with methyl green (6) showed beyond question the identity of many of the latter with the osmophile granules. The above-mentioned unstained sections were then soaked in warm xylol twenty-four hours and left mounted in xylol balsam until the osmophile granules had so nearly disappeared (at first the xylol merely removes the blackening caused by the osmic) that their presence could be detected only by comparing the sections with photographs which indicated exactly where to look for each granule. The sections were then stained with orange-methyl green, and the faded osmophile granules *selected* the green and became again distinctly visible. Other sections were stained with acid fuchsin before being mounted in glycerine, and these showed

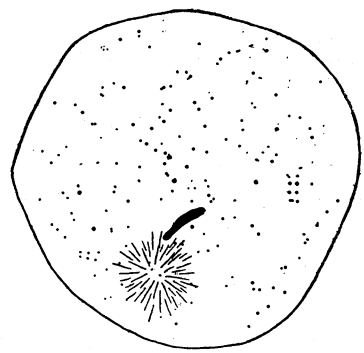


FIG. I. — Section of oöcyte, 2d order, showing one form of distribution of the osmophile granules. Osmophile granules in cytoplasm and sphere drawn with camera lucida. Rays diagrammatic.

a striking contrast of red nucleoli and centrosomes, with black osmophile granules.

One unfertilized oöcyte, first order, contained eleven nucleoli (?) distributed in and near the spindle and throughout the cytoplasm. The acid fuchsin was subsequently removed from these structures with 70% alcohol, and they could no longer be seen without the aid of sketches to indicate their position, while the black osmophile granules remained as sharply differentiated as before. In unstained preparations (such as described above) we have found these granules at all stages of the development of the egg, from the smallest oöcytes (or oögonia) to the segmenting ova, and in the former, one is occasionally found so exactly in the center of the yolk nucleus that in a stained preparation it would be unhesitatingly pronounced a centrosome. Tiny osmophile granules are often seen within the attraction spheres, spindle, and cones, though they are *far more* numerous throughout the rest of the cytoplasm. In some cases one, two, or more osmophile granules have been seen apparently in the exact center of a sphere (Text-fig. I). Throughout the rest of the cytoplasm, both their distribution and form vary greatly, even at exactly the same stage of the development of the egg. As to distribution, they are sometimes quite evenly distributed throughout the cytoplasm (Text-fig. I), and again large areas appear to be entirely free from them. As to size, they are sometimes tiny microsomes (Photo. 8 and Text-fig. I), and again many of them are as large and homogeneous as nucleoli (Photo. 12), while in many cases they appear as masses — aggregations of individual granules (Photo. 13). Whether any one of these conditions is distinctive of the normal egg we are unable to determine at present.

There are equally marked variations in their response to stains ; after a short immersion, iron haematoxylin removes the blackening caused by osmic fixatives — and, as a rule, it does not stain the granules. After prolonged immersion (three days) the osmophile granules show only a faint indication of the stain, and in these cases, when mounted in xylol balsam, they entirely dissolve out of the sections (*cf.* Photos. 13 and 14). In exceptional cases, however, they have formed an insoluble compound

with the stain and cannot be dissolved out. Photo. 9 is a section of an ovarian egg, in which these granules stained intensely with iron haematoxylin, all the sections in this slide giving the same reaction. The next slide of sections of the *same ovary* was treated similarly, and the osmophile granules did not stain.

In a few cases we have completed the entire process of killing, sectioning, staining, and mounting, within ten hours; and in these cases the granules have responded sharply to the stain, but we have not repeated this method often enough to test its value.

Formation of Vesicles between First and Second Maturation Spindles. — In earlier papers (4–6) one of us has stated that at the telophase of both the first and second maturation spindles, the chromosomes assume the form of small vesicles, corresponding in number to the number of the chromosomes.

Mead (8) has seen in *Chaetopterus* similar vesicles at the telophase of the second maturation spindle. He describes the formation of these as follows : "When the chromosomes have reached a position near the poles of the spindle, each of them swells up to form a vesicle, in which, at first, two distinct rows of granules may be seen. Later, each chromosome exactly resembles a miniature nucleus." Whether the chromosomes of the second maturation spindle of *Allolobophora foetida* form their vesicles in this way, we are unable to state, as we have not yet secured preparations of the second maturation spindle showing the metamorphosis of the chromosomes to vesicles. Of the first maturation spindle, however, we have preparations showing these transitional stages, and they suggest a method of formation differing from that of the second maturation spindle of *Chaetopterus*. Text-fig. II represents a number of forms assumed by the daughter-chromosomes of the tetrads of the first spindle. At the telophase of this spindle (Photo. 11) we find these forms both in the polar body and egg. They

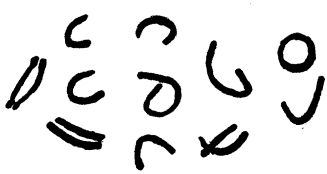


FIG. II. — Some forms shown by the chromosomes at the telophase of the first maturation spindle, showing the probable method of formation of the eleven vesicles, which are present a little later. Camera, Zeiss 2 mm. immer., oc. 12. *Cf.* Photo. 11.

suggest that the vesicles of this stage are formed by the two parts of each chromosome uniting to form a ring.

Living Cytoplasm. — The cytoplasm of the living egg of *Allolobophora foetida* presents a dissimilar structure at different stages of its development, and it has been our aim to demonstrate this difference and to determine, if possible, what structures in the sections can be identified with those seen in the living egg.

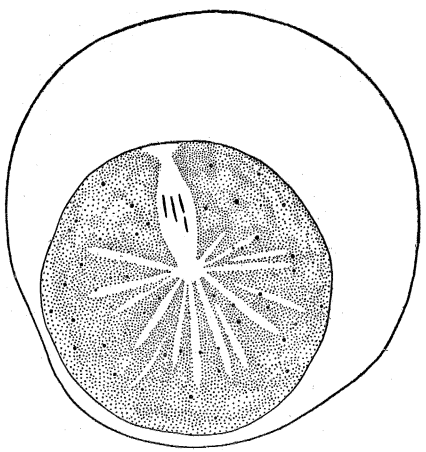


FIG. III. — Living unfertilized egg, from perfectly white cocoon. Only the larger osmophile granules represented. Chromosomes distinctly visible. Camera, 2 mm. immer., oc. 2.

In an egg at the pronuclear and first cleavage stages we have a definite cytoplasmic feature, which is not so pronounced in the less mature egg. This feature is conspicuously shown in Photo. 1 in the form of globules of varying sizes, which appear to be an approximately transparent non-miscible substance suggesting drops of sap or oil. We have designated them as sap globules rather than oil globules, as they do not blacken with osmic.

In the living oöcyte, first order, we have not been able to demonstrate this structure (Text-fig. III), but in oöcyte second order we find very tiny sap globules (Text-fig. IV) which gradually increase in size as the egg matures (Photos. 4–5, I); (the globules developing whether the egg is fertilized or not).

FIG. IV. — Peripheral sap globules after formation of 1st polar body. Traced from photographic negative of living egg. × 500.

In abnormal eggs the sap globules are relatively large — the early stages of a pathological condition being apparently expressed by a too rapid development of the cytoplasm. The normal enlargement of the globules, as expressed in later stages, cannot be due to individual growth, for the increase in the size of the egg is not at all commensurate with

the enlargement of the sap globules. It would seem, rather, that this sap, which in some form must also be present in oöcyte first order, is molded into the more definite shapes of the later stages by a rearrangement of the other constituents of the cytoplasm. If an egg is gently pressed until a tiny break is made in the outer membrane, the larger globules become somewhat constricted in form when flowing through this aperture as represented in Text-fig. V. After escaping from the pressure of the aperture, they regain their spherical shape.

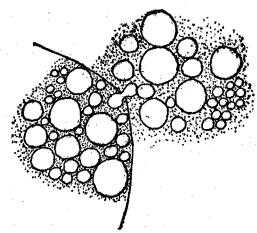


FIG. V. — Sap globules pressed out of living egg. (Pronuclear stage.) Free-hand sketch. To the right a large globule has broken into several small ones. One of the large globules above is the result of the fusing of two smaller ones.

When an egg at the pronuclear stage has been kept in water too long, the globules fuse and swell, many of them increasing in size two or three times their diameter, giving the egg a vacuolated appearance.

This rapid and definite response to abnormal conditions suggested to us their value as a guide to determine the relatively harmful effect of killing fluids, for any fixative producing a marked disturbance of the sap globules or the surrounding substance would probably cause the globules to fuse or break up at once. Our method has been as follows (under a Zeiss 2 mm.) : First, to focus on the periphery of an egg showing

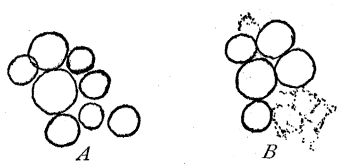


FIG. VI.

FIG. VII.

FIG. VI. — *A*. Sap globules of living egg. *B*. The same globules after 15 minutes in 1 per cent. osmic. Zeiss 2 mm. immer., oc. 2 (camera).

FIG. VII. — *A*. Sap globules of living egg. *B*. The same area after 15 minutes in chromo-acetic. Zeiss 2 mm. immer., oc. 2 (camera).

pronounced sap globules, sketching about half a dozen of these with the aid of the camera (Text-fig. VI, *A*) ; second, one of us then applies the fixative, while the other closely watches the effect produced on the sap globules ; third, after 15 to

30 minutes, the same globules are again sketched to illustrate the effect produced by the fixative (Text-fig. VI, *B*). A comparison of Text-figures VI and VII will show the relative injury to the form of the globules produced by 1% osmic acid and chromo-acetic. Photo. 3 further illustrates the effect of chromo-acetic on the sap globules and a comparison of this Photo. with Photos. 2 and 5 will show that osmic acid and corrosive sublimate are far less injurious. In Photo. 2 many of the sap globules in the center, and the line of globules on the right, were sketched from the living egg (camera lucida), and a comparison of the sketch with the photograph shows scarcely perceptible change in structure. Such changes as occur later in corrosive sublimate and osmic preparations are probably produced by the alcohols and imbedding. In order to test this we have fixed, stained, hardened, and cleared eggs under a Zeiss 2 mm. immer., oculars 2 and 4. We have repeated many times each step in the technique, selecting, as in the case when the fixative alone was tested, a definite number of sap globules upon which to center our attention. As, however, the egg becomes more opaque during the process of hardening, this method does not promise to be as satisfactory as a complete comparison of sections with freshly fixed material of the same stage. The above-mentioned method of applying the fixative while focusing on the periphery of the egg (under such gentle pressure that the egg continues to develop normally) has been supported by applying the fixative to a thinner layer of cytoplasm, obtained by gently pressing an egg until it is flattened to the outer membrane. The form of the sap globules remains unchanged under this pressure, and their reaction to the fixative appears to be the same as when the egg is not subjected to pressure. Whether any part of the sap globules becomes coagulated by the fixatives and takes part in forming the network seen in some sections we are unable to determine. If the globules are present in any form, they do not stain, for the vacuoles seen in the sections (Photos. 6, 16–18) undoubtedly answer to these structures. How far the breaking or fusing of the sap globules may be responsible for definite features of certain fixatives we are not prepared to answer until

we can support our conclusions with a larger number of photographs of sections.

In the living egg there appear to be at least four constituents of the cytoplasm.

1. The above-mentioned clear, approximately transparent sap globules of varying sizes (Photos. 1, 2, 4, 5).

2. Dense, opaque, deutoplasmic granules, varying in size from tiny points, scarcely visible under a magnification of one thousand diameters, to those plainly seen with the low powers. In form, size, and distribution they appear to answer to the above-mentioned osmophile granules (page 130) (Photos. 4, 5, 7–9, 12, 13, and Text-fig. I). These granules dance about with great activity in eggs that have been kept too long in artificial media — this abnormal activity being probably due to pathological disturbances in the rest of the cytoplasm.

3. Nucleolar-like bodies, strongly refractive in the living egg — that do not blacken with osmic. In the sections they react to the stains selected by the nucleoli of the pronuclei. The sperm-granules (4 and 6) react to the same stains.

4. Lighter areas which are relatively free from the sap globules and osmophile granules — these areas being represented by the polar rings, spindle, attraction sphere, and the interspaces of the sap globules. This substance does not blacken with osmic and appears distinctly granular in fixed eggs. It stains intensely, and in the sections appears more opaque than the rest of the cytoplasm — even in those cases where the osmophile granules are not dissolved or faded out. Thus the lighter areas of the living egg are the darker areas of the sections. (*Cf.* Text-fig. III and Photo. 17.) A study of the living egg suggests no fundamental difference between that part of those lighter areas which contributes to forming the polar rings, spindle, and sphere (archoplasm (5)), and the part occupying the interspaces of the globules. We have been able to demonstrate a difference by differential staining of the sections (5), thus far succeeding only with the double stains. We have, however, additional data on this point arguing strongly for the specific nature of archoplasm.

Chromosomes in the Living Egg. — The rarity of the cases in

which we have been able to see chromosomes within the spindle led us at first to think that the living eggs which showed this exceptional feature must be abnormal, for, as a rule, the chromosomes are not visible until the egg dies naturally, or is killed by a fixative. It was not until we were able to watch an egg develop normally after the chromosomes were seen, that we were convinced these exceptional cases were due to other causes. This does not appear to be wholly dependent upon the position of the spindle, for often in cases where the spindle is in the most favorable position, and clearly indicated, the chromosomes cannot be seen, even with the highest powers.

Text-fig. III represents a living oöcyte, first order, taken from a freshly deposited cocoon. The chromosomes at the metaphase were so distinct that two of them were readily traced with the camera. We watched this egg until it constricted off its first polar body, and then we killed it in chromo-acetic, stained and hardened it, and in every respect it appeared to be a normal oöcyte, second order. We would accentuate the fact that the chromosomes in the living egg showed exactly the same form as those seen in sections, as this possibly indicates that the chromosomes are less sensitive than the cytoplasm to the action of the fixatives. The fact that these centers of activity are more staple than the cytoplasm, one of us suggested in a former paper (7), where it was stated that the pronuclei continued to develop long after the cytoplasm was unquestionably abnormal.

The egg represented in Text-fig. III was below the average in size, thus transmitting relatively more light ; this fact probably accounting, in part, for the relatively distinct outlines of the structures within the egg. The broad, clear rays, which could be traced from the attraction sphere almost to the periphery, do not appear to correspond to the rays so clearly outlined in chromo-acetic sections (Photo. 15), but rather to those of osmic acid sections (Photo. 17). This photograph is technically poor, owing to the fact that it is taken from a section 10μ thick. It is introduced only because it is the best example we have (in sections) of a structure that can be compared to the rays of an attraction sphere in the living egg. We are not yet pre-

pared to discuss the finer details of these structures, for we feel we must wait until we can control the shrinkage of the eggs killed in osmic acid, before placing much confidence in the morphological details seen in sections. This shrinkage occurs in the alcohols. When formalin is substituted as a hardener, the shrinkage is much reduced, but the use of formalin prohibits sharp staining. The spinning phenomena, which has been seen by Mrs. Andrews (1) during the formation of the polar bodies in other eggs, we have not yet been able to detect, but in view of the exceptional cases in which we have seen the chromosomes and other details in the living egg of *Allolobophora foetida*, we are not prepared to say that the above-mentioned spinning phenomenon does not occur.

Shrinkage. — A comparison of the size of sections of eggs at a given stage with the size of the average living egg at the same stage shows that, at some point or points in the technique, a large amount of shrinkage has occurred, in some cases amounting to one-half the diameter of the living eggs. With a view to determine when the shrinkage occurs, we have first measured the living egg and then each step in the subsequent technique. In this manner we have tested twenty-eight fixatives, the compound fixatives and their component parts, each in varying strengths and varying the time the egg was immersed in the fixative from five minutes to twenty-four hours. An attempt to formulate the data gathered from these experiments has shown that the action of a given fixative upon eggs, even at the same stage of development, is extremely inconstant. But as a general rule, subject to many exceptions, it may be said: First, certain fixatives shrink the living egg, and in these cases relatively little shrinkage is produced by the subsequent treatment with the alcohols, *e.g.*, strong chromic acid and, in most cases, corrosive acetic (strong). Second, certain fixatives do not shrink the living egg, and in these cases they shrink more or less during the subsequent treatment with alcohols, *e.g.*, weak osmic acid, .1% to 1%, and corrosive sublimate. Third, certain fixatives swell the living egg, the subsequent treatment with alcohols producing a slight shrinkage — the final result being a mounted egg with almost the same diameter as the

living, *e.g.*, chromo-acetic,[1] strong osmic acid, platinum chloride. Fourth, the amount of shrinkage caused by the fixative is dependent upon the stage of development reached by the egg, the unfertilized egg being much more sensitive to the fixative.

The hundreds of eggs that we have measured have served merely to impress us with the fact of the inconstant effect of the fixatives and subsequent technique — this inconstancy speaking for the individuality of each egg. As shrinkage must be an important factor in determining the final distribution of the cytoplasmic elements, we hope to be able to collect enough data on this point to be of service.

PHOTOMICROGRAPHY.

Preface. — In the autumn of 1893 and the winter of 1894, my friend Dr. Charles G. Fuller, of Chicago, successfully photographed a full series of my sections of the egg of *Allolobophora foetida*, illustrating successive steps in the maturation and fertilization of the egg.[2]

The work was done with the Zeiss horizontal photomicrographic camera, Zeiss microscope with apochromatic condenser, Zeiss projection oculars 2 and 4, and Zeiss apochromatic lenses 16–2 mm. immer., 140 aperture. Artificial light was used. I am glad of this opportunity to express my indebtedness to Dr. Fuller. The good quality of his work will speak for itself when the photographs are published.

These photographs were shown at Woods Holl in the summer and early autumn of 1894, and, as far as I am aware, they were the first photomicrographs of sections showing the processes of maturation and fertilization of the egg.— KATHARINE FOOT.

Our work has been done with a Bausch and Lomb vertical photomicrographic camera, Zeiss microscope, apochromatic

[1] In 1896 (5) I regarded chromo-acetic as the most reliable fixative, giving as one reason for this, that eggs measured before killing, and after mounting, gave almost the same diameter. At that time I had not measured the eggs at *each step* in the *technique*, and the measurements were not extended to sections. — K. FOOT.

[2] As these photographs have no especial bearing on the details discussed in this preliminary, I shall reserve their publication for a future paper.

lenses 16–2 mm. immer., 140 aperture, Zeiss compensating and projection oculars. We have not used a magnification beyond one thousand, finding this will reproduce details that can be clearly seen only with a 2 mm. immer. and ocular 8.

With a 2 mm. immer. projection ocular 4, diaphragm of ocular at 0, and the longest draw, the magnification attained is nearly one thousand [about 960]. When the diaphragm of projection ocular 4 is adjusted to 10, the magnification is much less ; *e.g.*, a draw giving a magnification of 520, with the ocular adjusted at 10, will give a magnification of 670, with adjustment at 0. We tested our magnification by measuring the object with a Zeiss micrometer eye-piece, then taking the measure of the photograph in microns, and dividing the latter by the former. On account of the difference in magnification dependent upon the adjustment of the projection ocular, we found this the only accurate method. Light — clear daylight ; sun shining, but not on mirror. Time — as near noon as convenient. Exposure — as a rule, fifteen to thirty seconds for sections.[1]

It gives us pleasure to express our indebtedness to Prof. Henry Crew, of Northwestern University, for recommending to us the following methods of developing and printing, and for instruction in their use. Plates — Seed 27. Developer — Metol. Printing paper — Kirkland's Lithium.

We wish also to express our obligation to Mr. J. G. Hubbard for our first lessons in developing and printing.

The experiments of a year with photomicrography have convinced us of its utility as a practical aid in cytological investigation, and we hope in this paper to argue for its more general adoption. The impossibility of photographing fine cytological details, which can be readily illustrated by a drawing, has been urged by Flemming (3) and others as the principal argument against the use of the camera in cytological work, Wilson's atlas (9) and Erlanger's photographs (2) serving to support these objections.

Those who have attempted to photograph cytological details

[1] For photography by daylight it is necessary to have a time shutter in the camera.

realize the following technical difficulties. At a magnification of one thousand, which is often necessary to bring out these details, not enough light can be transmitted through our thinnest sections to admit of focusing delicate structures on the ground glass of the camera; *e.g.*, even with the aid of the best focusing glass the small centrosome shown in Photo. 15 cannot possibly be seen on the ground glass. It is barely possible to see this detail through the microscope with a 2 mm. immer. and ocular 8, and we were astonished to find it so distinctly reproduced in the photograph. The ring chromosomes in Photo. 11 further illustrate this point.

It is impossible, however, to focus such details on the ground glass, and it has been our aim to devise some method of overcoming this difficulty by discarding the ground glass as a factor in focusing.

Selecting a structure that could be clearly focused on the ground glass at a magnification of one thousand (a sharply stained nucleolus, for example), we first focused through the microscope, making a note of the exact position of the pointer on the face of the micrometer screw. We then slipped the camera down, focusing the nucleolus on the ground glass. The position of the pointer on the micrometer screw then indicated exactly the difference between the two foci. In order to facilitate the accurate measurement of this variation in focus we marked off into twenty parts each of the twenty-five divisions that are designated on the face of the micrometer screw. The difference in focus proved to be about $\frac{3}{20}$ of one of the twenty-five divisions; *e.g.*, with the pointer at the 5 mark for the focus through the microscope, to get the focus on the ground glass, turn the screw until the pointer indicates 4 and $\frac{17}{20}$.

We have tested the accuracy of this method by photographing at 960 diameters such a detail as the centrosome in Photo. 15. We took five photographs in as many minutes, keeping all the factors unchanged except the focus. One photograph was taken at what we calculated to be the correct focus; *i.e.*, $\frac{3}{20}$ above the focus through the microscope. Two were taken above this point and two below. Trying this several

times, we found that the variation of $\frac{3}{20}$ almost unfailingly caught the desired focus.

As two or three photographs can be taken in as many minutes, we generally take three of each preparation, one at the tested focus, one $\frac{1}{20}$ above, and one $\frac{1}{20}$ below this point, in case any difference in temperature, thickness of the cedar oil, or any other unforeseen factor should affect the focus. The few minutes required to develop these extra negatives is time well spent, for occasionally the focus above or below the tested one proves the best. This variation in focus can be just as readily settled for any magnification, and it does away entirely with the hopeless effort of attempting to focus fine details on the ground glass. We have tested the method with different magnifications and different lenses, and find it works admirably in all combinations, but it seems unnecessary to give figures for the different tests, as the variation is undoubtedly a point that must be settled for each microscope.

A method of work that can aid the biologist in seizing accurately and rapidly any points of interest his material offers, and enables him to retain them in a form convenient for comparative study, must certainly be of great value in the laboratory. Photomicrography appears to us to fill just such a need. A dozen photographs of a variety of features can be taken in the time required to reproduce any one of them by a careful drawing. The printed photographs can be kept in a form serviceable for frequent reference, and the impression first made by the preparation not allowed to fade. Possibly a photograph is less intelligible than a simplified sketch to any one unfamiliar with the preparation, but cannot this be said of the preparation itself? We have collected over two hundred sketches and as many photographs, illustrating features in our sections we wished to preserve for comparative study. Of the relative values of these two methods there can be no question, in every case the photographs proving to be the more valuable aid in recalling the preparations. We are not pleading to replace the sketch with the photograph, but we would argue for the use of both, letting the photograph speak for the preparation and the

sketch for the investigator's interpretation. One of the criticisms of Erlanger's photograph, most commonly heard, emphasizes the worthlessness of his preparations. Is not this the strongest possible argument in favor of photomicrography?

WOODS HOLL, October 10, 1898.

PAPERS REFERRED TO.

1. ANDREWS, GWENDOLEN FOULKE. Some Spinning Activities of Protoplasm in Starfish and Echinus Eggs. *Journ. of Morph.* Vol. xii, No. 2. 1897.
2. v. ERLANGER, R. Beiträge zur Kenntniss der Structur des Protoplasmas, der karyokinetischen Spindel und des Centrosoms. *Arch. f. mikr. Anat.* Bd. xlix. 1897.
3. FLEMMING, W. Zelle. *Ergebnisse der Anat. u. Entwicklungsgeschichte, Merkel u. Bonnet.* Bd. vi. 1897.
4. FOOT, KATHARINE. Preliminary Note on the Maturation and Fertilization of the Egg of Allolobophora foetida. *Journ. of Morph.* Vol. ix, No. 3. 1894.
5. ——— Yolk-nucleus and Polar Rings. *Journ. of Morph.* Vol. xii, No. 1. 1896.
6. ——— The Origin of the Cleavage Centrosome. *Journ. of Morph.* Vol. xii, No. 3. 1897.
7. ——— The Cocoons and Eggs of Allolobophora foetida. *Journ. of Morph.* Vol. xiv, No. 3. 1898.
8. MEAD, A. D. Some Observations on Maturation and Fecundation in Chaetopterus pergamentaceus Cuvier. *Journ. of Morph.* Vol. x, No. 1. 1895.
9. WILSON, E. B. An Atlas of the Fertilization and Karyokinesis of the Ovum. Macmillan & Co. New York, 1895.
10. ZIEGLER, HEINRICH ERNST. Untersuchungen über die ersten Entwicklungsvorgänge der Nematoden. *Zeit. f. wiss. Zool.* Bd. lx, Heft 3. 1895.

EXPLANATION OF PLATE A.

THE reproductions for the following plates were made by Edward Bierstadt of New York. His work was so admirably done that neither strength nor definition has been sacrificed by the process of reproduction.

In order to economize space, only a small portion of the negatives of Nos. 1–5, 10, 11, 13–15, has been reproduced. The eggs of Nos. 2–5, 10, were fixed under a Zeiss 2 mm. immer., one of us applying the fixative while the other watched its effects. All the photographs were taken with Zeiss 2 mm. immer. projection ocular 4.

PHOTO. 1. Living egg (stage, telophase of first cleavage spindle). Periphery of egg, showing part of a polar ring, surrounded by sap globules. Egg slightly colored with weak methyl green. The negative of this egg being a little sharper, it was chosen in place of one of an unstained egg of the same stage. In these cases the light was transmitted through a part of the egg fully 100 μ thick; thus a very sharp negative could scarcely be expected. × 500. Medium, distilled water.

PHOTO. 2. Periphery of a segmenting egg, after 20 minutes in corrosive sublimate. Delafield haematoxylin. Before killing the egg, many of the sap globules were sketched with the camera lucida, including the line of five on the right. The fixative produced no perceptible change in their size or contour. (*Cf.* No. 5.) × 500. Medium, distilled water.

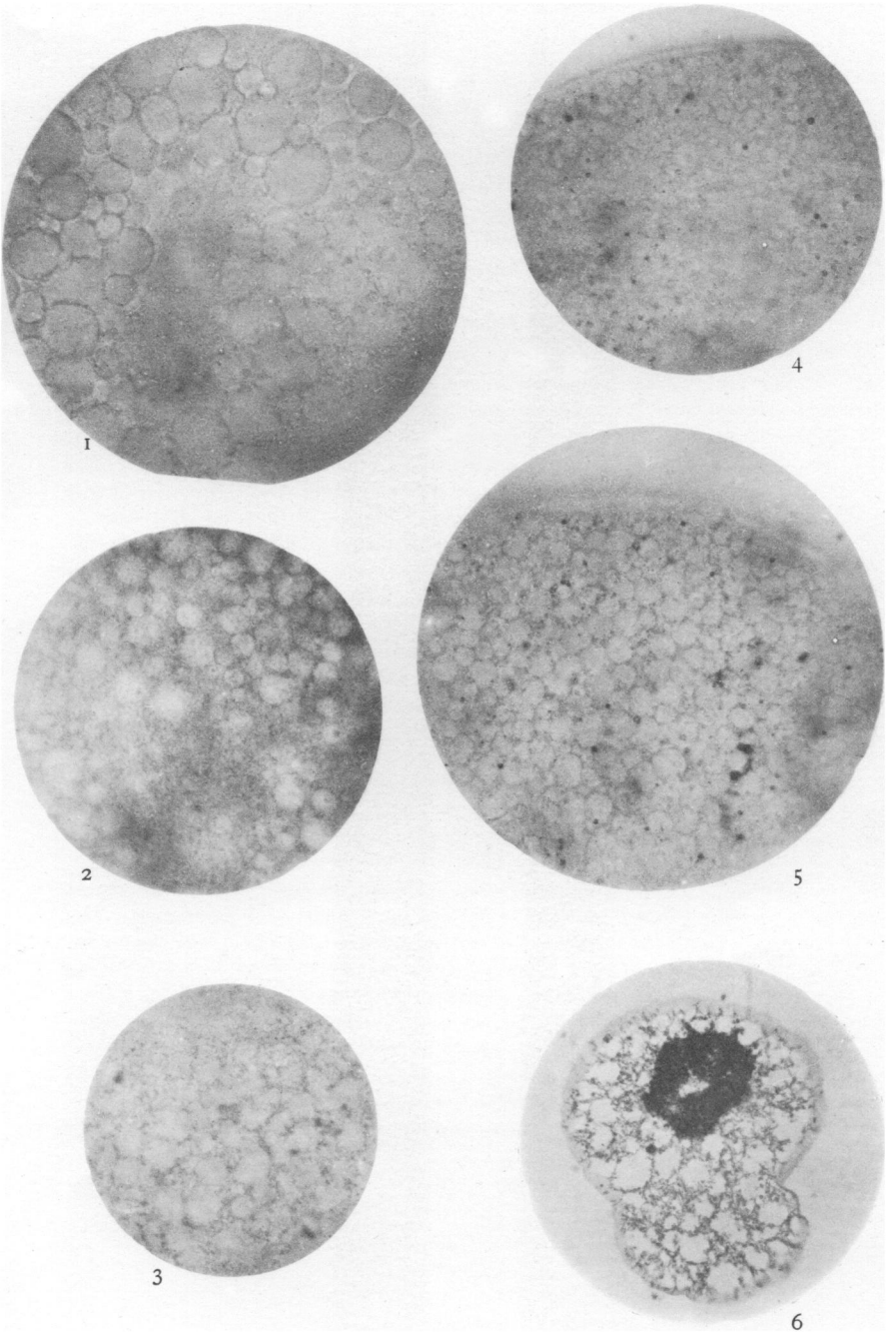
PHOTO. 3. Periphery of oöcyte, second order, after chromo-acetic (15 mm.). Stained with alum cochineal, hardened in alcohol and cleared in xylol. The small sap globules have fused, forming irregular patches. × 500. Medium, xylol.

PHOTO. 4. Periphery of egg just after formation of second polar body. Slightly flattened. Killed in .1 % osmic acid (15 mm.). Gentian violet. × 500. Medium, distilled water. *Cf.* size of sap globules with those of egg of a little later stage (No. 5).

PHOTO. 5. Periphery of egg at pronuclear stage. Pronuclei one-half maximum growth. Egg pressed until the cytoplasm reached the outer membrane. This was done under a 2 mm. immer., and the sap globules appeared neither broken nor fused by the gentle pressure. A comparison of the photograph with a living egg at the same stage shows them to be the normal size. 1% osmic acid, 1 hour. Unstained. × 500. Medium, distilled water. The sharp black specks are osmophile granules; those out of focus appear as faint rings.

PHOTO. 6. Section (4 μ) through cytoplasm and one polar-ring of egg at telophase of first cleavage spindle. Fixative, chromo-acetic. Hardened in 40 % formaldehyde, 26 hours. Stain, iron hæmatoxylin. × 340. Medium, xylol balsam.

PLATE A.



PHOTOGRAPHS BY FOOT AND STROBELL.

ARTOTYPE, E. BIERSTADT.

EXPLANATION OF PLATE B.

PHOTO. 7. A small piece of the cytoplasm of an oöcyte, second order. Unstained. The egg was killed in 1% osmic, and after one hour crushed on the slide. This was to demonstrate the presence of the osmophile granules to compare with those of No. 9. × 900. Medium, distilled water.

PHOTO. 8. Ditto. Changing focus in order to reproduce the three tiny granules, two on the left and one on the right of the preparation. In No. 7 the two on the left (out of focus) appear as tiny faint rings.

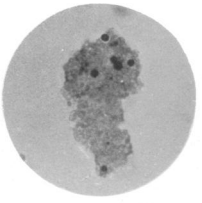
PHOTO. 9. Section (3 μ) through the cytoplasm of an ovarian egg at free end of ovary. Granular aggregations of archoplasm and osmophile granules, such as are found in the oögonia or tiniest oöcytes, and in eggs of later stages, both in the ovary and cocoon. *Cf.* Nos. 4, 5, 7, 8, 10, 12, 13. Fixative, chromo-acetic followed by osmic. Hardened in alcohol, 24 hours. Iron haematoxylin. × 860. Medium, xylol balsam.

PHOTO. 10. Nucleus in a macromere of a segmenting egg, showing two nucleoli and chromatic thread. Fixed under Zeiss 2 mm. immer., 1% osmic acid. Delafield haematoxylin. Exposure 1½ minutes, as the light was transmitted through the entire egg. × 900. Medium, distilled water.

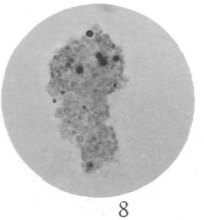
PHOTO. 11. Section (3 μ) of telophase of first maturation spindle. *Cf.* Text-fig. II. Fixative, chromo-acetic. Hardened in 5% formaldehyde, 48 hours. Iron haematoxylin. × 860. Medium, xylol balsam.

PHOTO. 12. Section (3 μ) of oöcyte, second order. Killed in chromo-acetic, washed in water, followed by 1% osmic acid for 30 minutes, to differentiate the osmophile granules. Hardened in alcohol. Unstained. × 500. Medium, glycerine. *Cf.* Text-fig. I. Attraction sphere and the refractive sperm indicated, although the egg is unstained.

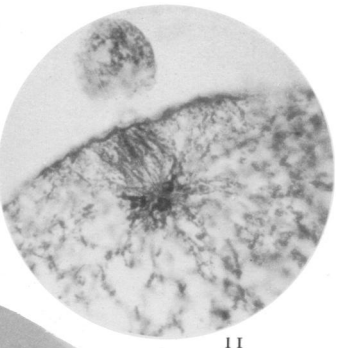
PLATE B.



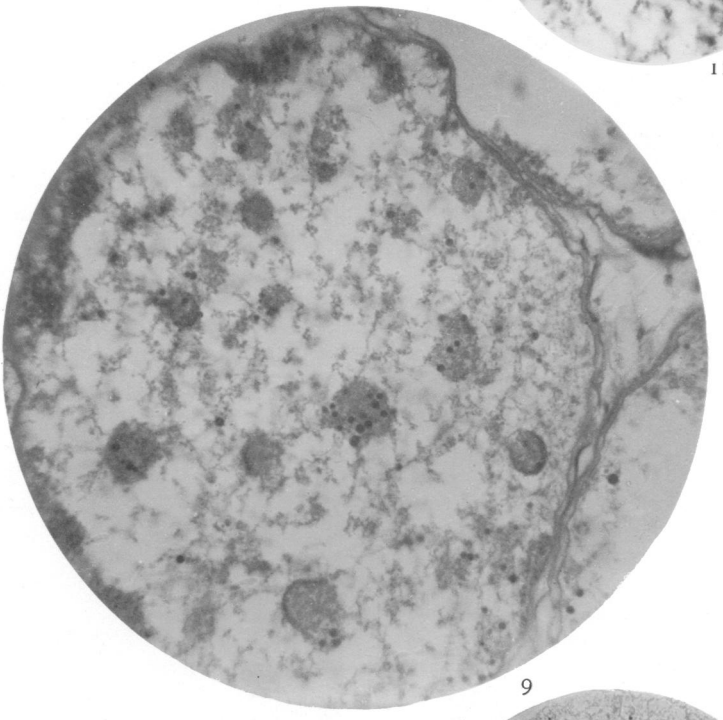
7



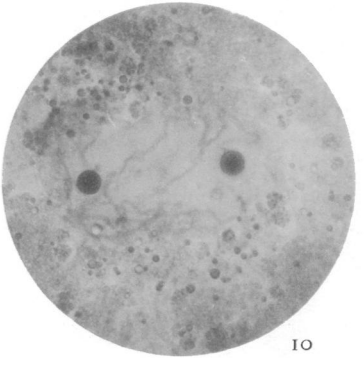
8



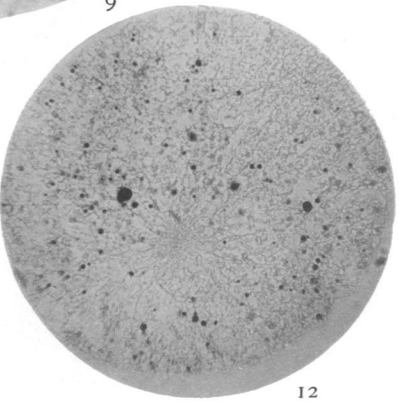
11



9



10



12

PHOTOGRAPHS BY FOOT AND STROBELL.

ARTOTYPE, E. BIERSTADT.

EXPLANATION OF PLATE C.

PHOTO. 13. Section (4 μ) through cytoplasm of oöcyte, second order. About one-third of the negative reproduced. Fixative, Hermann. Hardened in 10% formaldehyde, 21 hours. Unstained. × 870. Medium, glycerine. The photograph was taken to demonstrate the two aggregations of osmophile granules, and the smaller ones throughout the cytoplasm. *Cf.* No. 14, which is the same section, after staining in iron haematoxylin 24 hours, and dissolving out the osmophile granules with xylol and xylol balsam. A careful comparison of the two photographs will show that they are focused on the same plane, the stained preparation, however, showing no trace of the black granules demonstrated in the unstained section. No. 14 shows that the stain has differentiated certain granules that are not seen at all, or are very faintly indicated in the unstained preparation The section was finally stained deeply with methyl green, with the view of deter mining whether any granules could be differentiated within the vacant places formerly occupied by the black osmophile granules. None could be seen.

PHOTO. 14. See No. 13.

PHOTO. 15. Section (4 μ) through upper pole of first maturation spindle. A centrosome in the sphere, and equally sharply stained granules beyond the sphere. Fixative, chromo-acetic. Hardened in alcohol. Iron haematoxylin. × 960. Medium, xylol balsam.

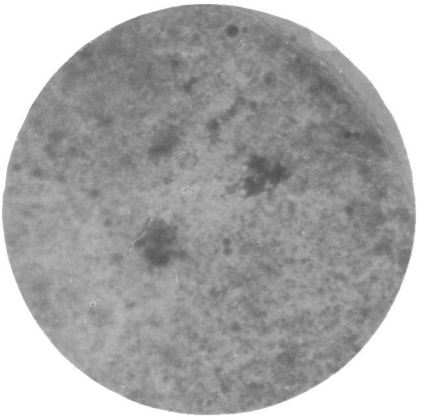
PHOTO. 16. Section (3 μ)[1] of egg at pronuclear stage, showing a part of one of the pronuclei which have attained about one-half their maximum growth. (After other fixatives, the pronuclei have a very different appearance.) Aggregations of archoplasm (polar-ring substance) at periphery; possibly some of the same substance around the pronucleus. Fixative, .2% osmic. Hardened in 5% formaldehyde, 22 hours. Iron haematoxylin, 28 hours. × 540. Medium, xylol balsam.

PHOTO. 17. A very thick section (10 μ) of an oöcyte, first order. Lower pole of first maturation spindle, and an indication of the chromosomes which are approaching the lower pole (spindle at anaphase). This section was photographed merely because it showed thick rays from the attraction sphere, which strongly suggest those seen in the living egg (represented in Text-fig. III, at a little earlier stage). The sap globules in this egg are larger than is the rule in normal eggs at this stage. No. 18 is the following section photographed to show the row of sap globules completely surrounding the attraction sphere, and to compare this attraction sphere with that of Photograph 15, in which case the egg was killed in chromo-acetic. Fixative, 1% osmic acid. Hardened in alcohol. Iron haematoxylin. × 500. Medium, xylol balsam.

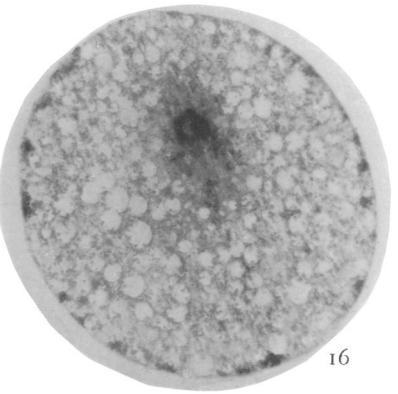
PHOTO. 18. See No. 17.

[1] We have tested the accuracy of our microtome by measuring the diameter of the eggs, both before and after sectioning, and counting the number of sections; *e.g.*, this section is one of a series of thirty, and the largest of these measures 94 μ in diameter.

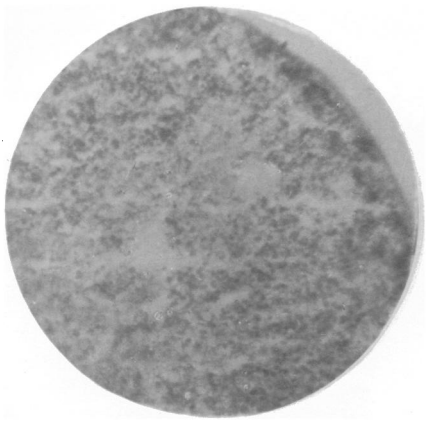
PLATE C.



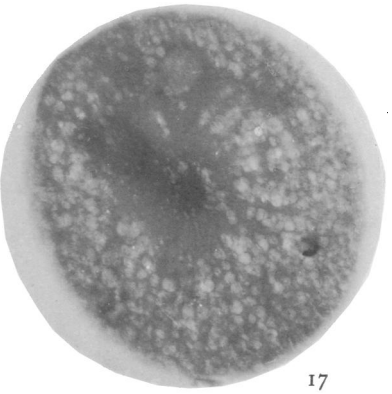
13



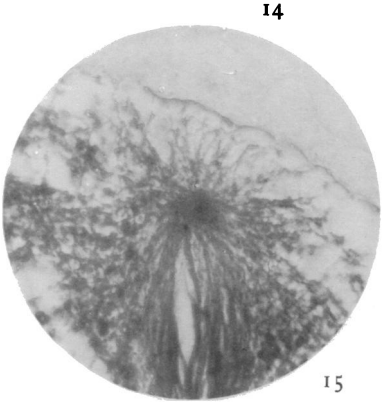
16



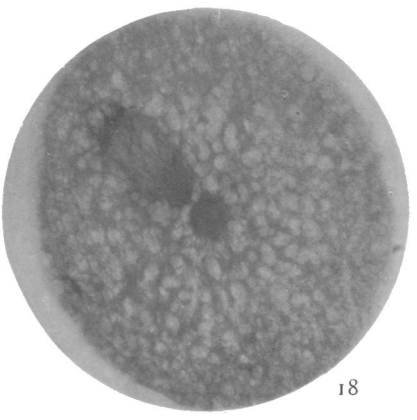
14



17



15



18

PHOTOGRAPHS BY FOOT AND STROBELL.

ARTOTYPE, E. BIERSTADT.